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(71) Applicant *(for all designated States except US)*: MALLINCKRODT MEDICAL, INC. [US/US]; 675 McDonnell Boulevard, P.O. Box 5840, St. Louis, MO 63134 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: MÄCKE, Helmut [DE/DE]; Kantonsspital Basel, Petersgraben 4, CH-4031 Basel (CH).

(74) Agents: BOONE, Jeffrey, S. et al.; Mallinckrodt Medical, Inc., 675 McDonnell Boulevard, P.O. Box 5840, St. Louis, MO 63134 (US).

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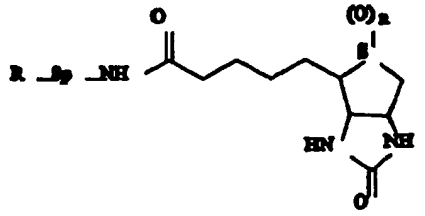


(I)

(57) Abstract

The invention relates to a labelled biotin compound, wherein said biotin compound is represented by general formula (I) wherein: n is 1 or 2, R is a chelating group for chelating a metal atom, and Sp is a spacing group having at least 4 atoms spacing NH from R; and wherein said biotin compound is labelled with a metal atom selected from (a) the group consisting of the radioactive isotopes ^{99m}Tc, ^{203}Pb, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{72}As, ^{111}In, ^{113m}In, ^{114m}In, ^{97}Ru, ^{62}Cu, ^{64}Cu, ^{52}Fe, ^{52m}Mn, ^{51}Cr, ^{186}Re, ^{188}Re, ^{77}As, ^{90}Y, ^{67}Cu, ^{169}Er, ^{117m}Sn, ^{121}Sn, ^{127}Te, ^{142}Pr, ^{143}Pr, ^{198}Au, ^{199}Au, ^{149}Tb, ^{161}Tb, ^{109}Pd, ^{165}Dy, ^{149}Pm, ^{151}Pm, ^{153}Sm, ^{157}Gd, ^{166}Ho, ^{172}Tm, ^{169}Yb, ^{175}Yb, ^{177}Lu, ^{105}Rh and ^{111}Ag or (b) the group consisting of the paramagnetic metal atoms Cr, Mn, Fe, Co, Ni, Cu, Pr, Nd, Sm, Yb, Gd, Tb, Dy, Ho and Er; said metal atom being attached to the biotin compound by means of said chelating group. The invention further relates to a pharmaceutical composition comprising said labelled biotin compound, to the use of said composition for diagnosis and therapy, and to a kit for preparing a radiopharmaceutical composition.

(RADIO) LABELLED BIOTIN DERIVATIVES

The present invention relates to labelled biotin compounds, to a method of preparing these compounds, to a pharmaceutical composition comprising these compounds, to the use of this composition for diagnosis and therapy, and to a kit for preparing a radiopharmaceutical composition.

It is well-known in the art, that various polypeptides or proteins, such as monoclonal antibodies (MAb's), bind with high affinity to tumour associated antigens. Consequently, radiolabelled monoclonal antibodies (MAb"s), have been successfully used for the in vivo localization of tumours in nuclear medicine. One of the major drawbacks in the use of MAb"s is their prolonged blood clearance. As a consequence, poor tumour/background ratios are usually achieved and the radiation dose to normal tissues is high.

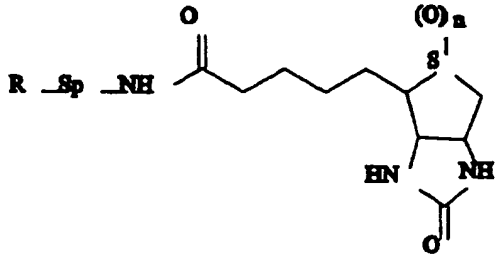
A promising development in the application of MAb"s in nuclear diagnosis is the pretargeting approach. According to this method the administration of the tumour-specific protein (antibody) and of the radioactivity take place at separate time points. This methodology is possible by use of the avidin/biotin system. There are two different basic protocols which can be used for tumour localization wih this avidin/biotin pretargeting system (Paganelli et al., Nucl. Med. Commun. 12, 1991, 211-234), viz. the two-step approach and the three-step approach. In the two-step approach an in vitro prepared conjugate of (strept)avidine and the tumour-seeking polypeptide, e.g. the antibody, is injected first, two or three days later followed by injection with radiolabelled biotin. By the three-step pretargeting approach, biotinylated MAb (1st step), streptavidin (2nd step) and radiolabelled biotin (3rd step) are injected successively (in about 24 h time intervals) to the patient. The first two steps, as well as the first step in the two-step approach, result in the avidination of the tumour. In addition, the biotinylated MAb's, which have not localized on the tumour but are still circulating in the blood, are macroaggregated by the excess of avidin. These avidin/MAb adducts of high molecular weight are rapidly taken up and catabolized by the liver. The 3rd step involves the in vivo administration of radiolabelled biotin. Due to the fast blood clearance of the non-tumour-bound biotin derivative, good tumour/background ratios are expected to be reached rapidly.

For the visualization of tumours, biotin, labelled with a radionuclide, preferably a metal-radionuclide, or with a paramagnetic metal ion are required. Since biotin is lacking

proper functional groups for stable metal binding, a suitable bifunctional ligand has to be coupled to biotin to enable labelling with the desired metal atom. Radiometal-labelled biotin derivatives are described in literature, e.g. in U.S. patent 5,283,342, in WO 93/25240, and by Koch and Mäcke (Angew. Chemie, Int. Ed. 31, 1992, 1507-1509). Vizzi et al. (J. Nucl. Med. 32, 1991, 920 – Proc. 39th. Ann. Mt., no. 403) have evaluated 12 different biotin derivatives labelled with technetium-99m. It is the conclusion of these authors, that the Tc-99m labelled biotin derivatives investigated show instability in vivo, as a consequence of which high levels of Tc-99m are found in normal tissues.

It is the objective of the present invention to provide a labelled biotin compound which has a high affinity to avidin, comparable with that of biotin itself, and which has an improved stability in vivo, i.e. an improved resistance to enzymatic breakdown, to allow its proper use in diagnosis and therapy.

This objective can be achieved by a labelled biotin compound, wherein said biotin compound is represented by the general formula



(I)

wherein:

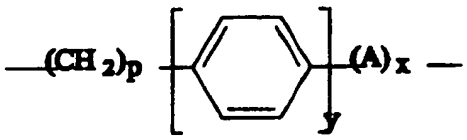
n is 1 or 2,

R is a chelating group for chelating a metal atom, and

Sp is a spacing group having at least 4 atoms spacing NH from R; and

wherein said biotin compound is labelled with a metal atom selected from (a) the group consisting of the radioactive isotopes ^{99m}Tc, ^{203}Pb, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{72}As, ^{111}In, ^{113m}In, ^{114m}In, ^{97}Ru, ^{62}Cu, ^{64}Cu, ^{52}Fe, ^{52m}Mn, ^{51}Cr, ^{186}Re, ^{188}Re, ^{77}As, ^{90}Y, ^{67}Cu, ^{169}Er, ^{117m}Sn, ^{121}Sn, ^{127}Te, ^{142}Pr, ^{143}Pr, ^{198}Au, ^{199}Au, ^{149}Tb, ^{161}Tb, ^{109}Pd, ^{165}Dy, ^{149}Pm, ^{151}Pm, ^{153}Sm, ^{157}Gd, ^{166}Ho, ^{172}Tm, ^{169}Yb, ^{175}Yb, ^{177}Lu, ^{105}Rh and ^{111}Ag or (b) the group consisting of the paramagnetic metal atoms Cr, Mn, Fe, Co, Ni, Cu, Pr, Nd, Sm, Yb, Gd, Tb, Dy, Ho and Er; said metal atom being attached to the biotin compound by means of said chelating group.

The above-defined spacing group Sp is preferably a group of the general formula



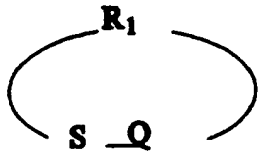
(II)

wherein A is a biradical of the formula

-NH-$(CH_2)_m$- or -NH-C(=X)-$(CH_2)_x$-Y-$(CH_2)_m$-C(CO_2H)H-

wherein **x** and **y** are each independently 0 or 1;
m is an integer from 1 to 4;
p is an integer from 0 to 4;
X is O or S; and
Y is NH, CO or S.

The above-defined chelating group R is preferably selected from the group consisting of $N_tP_qS_{(t+q)}$ tetradentate chelating agents, wherein t+q=2-4, or groups derived from ethylene diamine tetra-acetic acid (EDTA), diethylene triamine penta-acetic acid (DTPA), cyclohexyl 1,2-diamine tetra-acetic acid (CDTA), ethyleneglycol-0,0'-bis(2-aminoethyl)-N,N,N',N'-tetra-acetic acid (EGTA), N,N-bis(hydroxybenzyl)-ethylenediamine-N,N'-diacetic acid (HBED), triethylene tetramine hexa-acetic acid (TTHA), 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tri and tetra-acetic acid (DO3A and DOTA), 1,4,7,10-tetraazacyclotridecane-N,N',N'',N'''-tri and tetraacetic acid (TRI3A and TRITA), hydroxyethyldiamine triacetic acid (HEDTA), 1,4,8,11-tetra-azacyclotetradecane-N,N',N'',-N'''-tetra-acetic acid (TETA), substituted DTPA, substituted EDTA, or from deferoxamine (desferrioxamine), or from a compound of the general formula

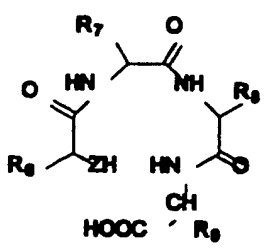


(III)

wherein R_1 is a branched or non-branched, optionally substituted
hydrocarbyl radical, which may be interrupted by one or more hetero-atoms selected from N, O and S and/or by one or more NH groups, and

Q is a group which is capable of reacting with an amino group of the peptide and which is preferably selected from the group consisting of carbonyl, carbimidoyl, N-(C_1-C_6)alkylcarbimidoyl, N-hydroxycarbimidoyl and N-(C_1-C_6)alkoxycarbimidoyl.

Suitable examples of $N_iP_qS_{(4+q)}$ tetradentate chelating agents are selected from



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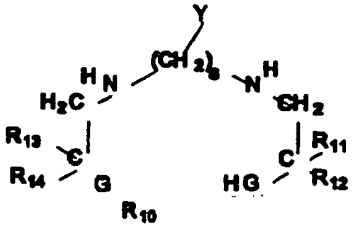

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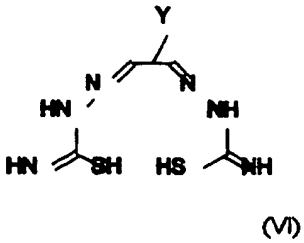

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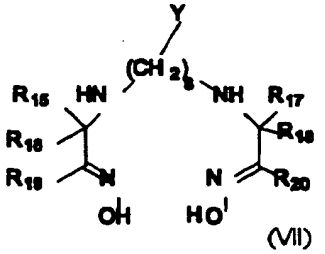

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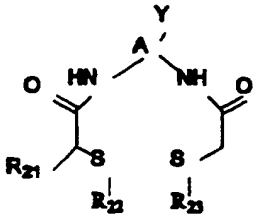
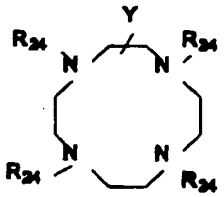


(VIII)



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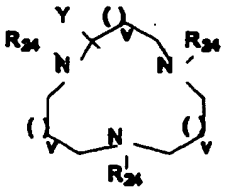

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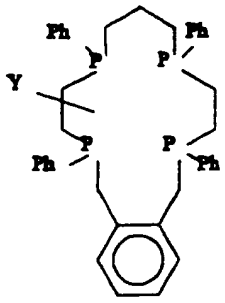

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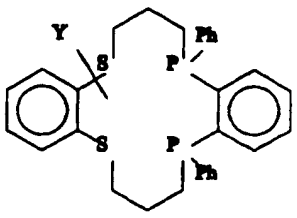
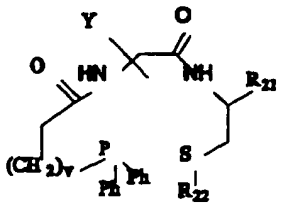


(XII)



(XIII)

wherein:

R_6-R_{20} are each individually hydrogen atoms or (C_1-C_4)alkyl groups, with the proviso that at least one of C_6 to C_9 is the symbol Y;

R_{21} is a hydrogen atom or a CO_2(C_1-C_4)alkyl group;

R_{22} and R_{23} are each individually (C_1-C_4)alkylcarbonyl, benzoyl or benzyl groups;

v is 0 or 1;

s is 2 or 3;

R_{24} is CH_2COOH or a functional derivative thereof;

A is (C_1-C_4)alkylene, if desired substituted with CO_2alkyl, $CH_2COalkyl$, $CONH_2$, $CONHCH_2CO_2$alkyl; phenylene, phenylene substituted by CO_2alkyl, wherein the alkyl groups have 1 to 4 carbon atoms;

G is NH or S;

Y is a valence bond or a functional group capable of binding with the spacing group;

and Z is S or O.

If Y is a functional group, Y preferably comprises isocyanato, isothiocyanato, formyl, o-halonitrophenyl, diazonium, epoxy, trichloro-s-triazinyl, ethyleneimino, chlorosulfonyl, alkoxycarbimidoyl, (substituted or unsubstituted) alkylcarbonyloxycarbonyl, alkylcarbonylimidazolyl, succinimido-oxycarbonyl, which group is prferably attached to a (C_1-C_{10})hydrocarbon biradical.

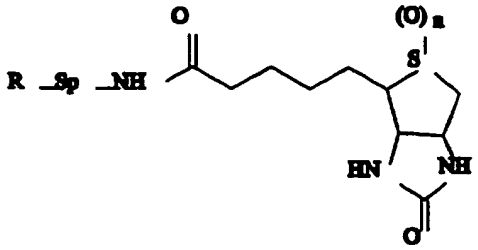
Suitable examples of hydrocarbon biradicals are biradicals derived from benzene, (C_1-C_4)alkanes, (C_2-C_6)alkenes and (C_1-C_4)alkylbenzenes.

Examples of suitable chelators of the general formula III are described in the international patent application WO 89/07456, such as unsubstituted or substituted 2-iminothiolanes and 2-iminothiacyclohexanes, in particular 2-imino-4-mercaptomethylthiolane.

The above labelled biotin compounds have been tested in a number of suitable model experiments that are predictive for in vivo application. These experiments are described in the Examples. From the results of these experiments it will be evident, that the labelled biotin compounds of the present invention have properties which make them suitable for diagnostic and therapeutic purposes. If labelled with a suitable atom for diagnostic purposes, the labelled biotin compound remains sufficiently long intact after administration to permit imaging of the target tumour without presenting a disturbing background. If labelled with a suitable radioisotope for therapy, such-labelled biotin compounds are promising therapeutic agents for the treatment of a number of malignant tumours.

The new metal-labelled biotin compounds of the invention can be prepared in a manner known per se for related compounds. For this purpose the biotin molecule is derivatized with the desired chelating agent as defined hereinbefore, e.g. a $N_tP_qS_{(q+q)}$ tetradentate chelating agent, or EDTA,

DTPA, etc., after introduction of the spacing group Sp as defined above, after which the compound obtained, having the general formula



(I)

wherein:

n is 1 or 2,

R is a chelating group for chelating a metal atom, and

Sp is a spacing group having at least 4 atoms spacing NH from R;

is reacted with a metal, as defined hereinbefore, in the form of a salt or of a chelate bound to a comparatively weak chelator, in order to form a complex.

Suitable examples of salts or chelates of the desired metal are: ^{111}In-citrate and acetate, ^{99m}Tc-tartrate, etc. The complex-forming reaction can generally be carried out in a simple manner and under moderate conditions.

The invention also relates to a biotin compound of the general formula I, as defined above, which compound can be used for the above-described method of preparing the labelled biotin compound.

The present invention further relates to a pharmaceutical composition, comprising in addition to a pharmaceutically acceptable carrier material and, if desired, at least one pharmaceutically acceptable adjuvant, as the active substance a labelled biotin compound as defined hereinbefore.

The invention also relates to a method for detecting and localizing tumours in the body of a warm-blooded living being, which comprises (i) administering to said being a composition comprising (strept)avidin conjugated with a polypeptide having a selective affinity to said tumour, (ii) thereupon, after avidination of said tumour, administering to

said being a composition comprising, in a quantity sufficient for external imaging, a labelled biotin compound as defined hereinbefore, said biotin compound being labelled with (a) a radioactive metal isotope selected from the group consisting of ^{99m}Tc, ^{203}Pb, ^{67}Ga, ^{68}Ga, ^{72}As, ^{111}In, ^{113m}In, ^{97}Ru, ^{62}Cu, ^{64}Cu, ^{52}Fe, ^{52m}Mn and ^{51}Cr, or (b) with a paramagnetic metal atom selected from the group consisting of Cr, Mn, Fe, Co, Ni, Cu, Pr, Nd, Sm, Yb, Gd, Tb, Dy, Ho and Er, and (iii) finally subjecting said being to external imaging to determine the targeted sites in the body of said being in relation to the background activity.

Instead of the above two-step protocol, the three-step pretargeting method can be used as discussed hereinbefore. In this method, the following protocol is used: (i-a) administering to said being a composition comprising a biotinylated polypeptide having a selective affinity to said tumour, (i-b) then administering a composition comprising (strept)avidin; both steps in substitution for the above step (i).

The invention also relates to a method of intraoperatively detecting and localizing tumours in the body of a warm-blooded living being, which comprises (i) administering to said being a composition comprising (strept)avidin conjugated with a polypeptide having a selective affinity to said tumour, (ii) thereupon, after avidination of said tumour, administering to said being a composition comprising, in a quantity sufficient for detection by a gamma detecting probe, a labelled biotin compound as defined hereinbefore, said biotin compound being labelled with ^{161}Tb, and (iii) finally, after allowing the active substance to be bound by and taken up in said tumour and after blood clearance of radioactivity, subjecting said being to a radioimmunodetection technique in the relevant area of the body of said being, by using a gamma detecting probe.

The above radioisotope, viz. ^{161}Tb, allows the use of a such-labelled peptide compound in the technique of radioguided surgery, wherein relevant tissues in the body of a patient can be detected and located intraoperatively by means of a gamma detecting probe. The surgeon can, intraoperatively, use this probe to find the lesions in which uptake of the compound labelled with said radioisotope, which is a low-energy gamma photon emittor, has taken place.

It will be clear, that instead of the above two-step protocol, the three-step pretargeting method can be used as discussed hereinbefore.

The biotin compounds of the present invention, provided they are radiolabelled with isotopes suitable for such purpose, can be used for the therapeutic treatment of tumours. So the invention further relates to a method for the therapeutic treatment of tumours in

the body of a warm-blooded living being, which comprises (i) administering to said being a composition comprising (strept)avidin conjugated with a polypeptide having a selective affinity to said tumour, (ii) thereupon, after avidination of said tumour, administering to said being a composition comprising,, in a quantity effective for combating or controling tumours, a labelled biotin compound as defined hereinbefore, said biotin compound being labelled with a metal isotope selected from the group consisting of ^{114m}In, ^{186}Re, ^{188}Re, ^{77}As, ^{90}Y, ^{66}Ga, ^{67}Cu, ^{169}Er, ^{117m}Sn, ^{121}Sn, ^{127}Te, ^{142}Pr, ^{143}Pr, ^{198}Au, ^{199}Au, ^{149}Tb, ^{161}Tb, ^{109}Pd, ^{165}Dy, ^{149}Pm, ^{151}Pm, ^{153}Sm, ^{157}Gd, ^{159}Gd, ^{166}Ho, ^{172}Tm, ^{169}Yb, ^{175}Yb, ^{177}Lu, ^{105}Rh and ^{111}Ag.

It will be clear, that instead of the above two-step protocol, the three-step pretargeting method can be used as discussed hereinbefore.

In case a radioactive labelled biotin compound is used as a diagnostic agent, it is frequently impossible to put the ready-for-use composition at the disposal of the user, in connection with the often poor shelf life of the radiolabelled compound and/or the short half-life of the radionuclide used. In such cases the user will carry out the labelling reaction with the radionuclide in the clinical hospital or laboratory. For this purpose the various reaction ingredients are then offered to the user in the form of a so-called "kit". It will be obvious that the manipulations necessary to perform the desired reaction should be as simple as possible to enable the user to prepare from the kit the radioactive labelled composition by using the facilities that are at his disposal. Therefore the invention also relates to a kit for preparing a radiopharmaceutical composition.

Such a kit according to the present invention may comprise (a) a composition comprising (strept)avidin conjugated with a polypeptide having a selective affinity to tumours, (b) a biotine compound of the general formula I, shown above, wherein the symbols have the above meanings, to which compound, if desired, an inert pharmaceutically acceptable carrier and/or formulating agents and/or adjuvants is/are added, (c) a solution of a salt or chelate of a metal isotope selected from the group consisting of ^{203}Pb, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{72}As, ^{111}In, ^{113m}In, ^{114m}In, ^{97}Ru, ^{62}Cu, ^{99m}Tc, ^{186}Re, ^{188}Re, ^{64}Cu, ^{52}Fe, ^{52m}Mn, ^{51}Cr, ^{77}As, ^{90}Y, ^{67}Cu, ^{169}Er, ^{117m}Sn, ^{121}Sn, ^{127}Te, ^{142}Pr, ^{143}Pr, ^{198}Au, ^{199}Au, ^{149}Tb, ^{161}Tb, ^{105}Pd, ^{165}Dy, ^{149}Pm, ^{151}Pm, ^{153}Sm, ^{157}Gd, ^{166}Ho, ^{172}Tm, ^{169}Yb, ^{175}Yb, ^{177}Lu, ^{105}Rh and ^{111}Ag, and (d) instructions for use with a prescription for reacting the ingredients present in the kit.

Preferably the biotin compound to be used as an ingredient of the above kit has been modified by a spacing group Sp and a chelating agent R as defined hereinbefore. The resulting biotin compound provides a facility for firmly attaching the radionuclide in a simple manner. Suitable chelating agents for modifying the biotin molecule are

described in detail hereinbefore. $N_pP_qS_{(4+q)}$ tetradentate chelating agents or N-containing di- or polyacetic acids or their derivatives, such as the compounds mentioned before, have proved to be pre-eminently suitable for attaching various metal radionuclides, such as In-111 and In-113m, to the biotin molecule. The kit to be supplied to the user may also comprise the ingredient(s) defined sub (a) and (b) above, together with instructions for use, whereas the solution of a salt or chelate of the radionuclide, defined sub (c) above, which solution has a limited shelf life, may be put to the disposal of the user separately.

The above kit is destined for use in a two-step protocol. If, instead thereof, the three-step pretargeting method is to be used, as discussed hereinbefore, the following kit is suitable: (a-i) a composition comprising a biotinylated polypeptide having a selective affinity to said tumour, and (a-ii) a composition comprising (strept)avidin; both compositions in substitution for the above composition (a).

In case the kit serves to prepare a radiopharmaceutical composition labelled with Tc-99m, Re-186 or Re-188, such a kit according to the present invention may comprise, in addition to the ingredient(s) defined sub (a) and (b) above, (c) a reducing agent and, if desired, a chelator, and (d) instructions for use with a prescription for reacting the ingredients of the kit with Tc-99m in the form of a pertechnetate solution, or with Re-186 or Re-188 in the form of a perrhenate solution. If desired, certain ingredients of the kit may be combined, provided they are compatible. The pertechnetate or perrhenate solution can simply be obtained by the user from a suitable generator.

When the radionuclide is present in the kit itself, the complex forming reaction with the biotin compound of the general formula I above can simply be produced by combining the components in a neutral or buffered medium and causing them to react. For that purpose the radionuclide may be presented to said biotin compound in the form of a chelate bound to a comparatively weak chelator, as described hereinbefore.

When the kit comprises a biotin compound as defined hereinbefore and is intended for the preparation of a radiopharmaceutical composition, labelled with Tc-99m, Re-186 or Re-188, the radionuclide will preferably be added separately in the form of a pertechnetate or perrhenate solution. In that case the kit will comprise a suitable reducing agent and, if desired, a chelator, the former to reduce the pertechnetate or the perrhenate. As a reducing agent may be used, for example, a dithionite or a metallic reducing agent. As a reducing agent for the above-mentioned kits is preferably used a metallic reducing agent, for example, Sn(II), Ce(III), Fe(II), Cu(I), Ti(III) or Sb(III); Sn(II) is

excellently suitable. Examples of suitable Sn(II)-compounds are $SnCl_2$, Sn(II)-tartrate, Sn(II)-phosphonate or -pyrophosphate, and Sn(II)-glucoheptonate. The modified biotin constituent of the above-mentioned kits, i.e. preferably the biotin compound, may be supplied as a solution, for example, in the form of a physiological saline solution, or in some buffer solution, or may be present in a dry condition, for example, in a lyophilized condition. When used as a component for an injection liquid it should be sterile, in which, when the constituent is in the dry state, the user should preferably use a sterile physiological saline solution as a solvent. If desired, the above-mentioned constituent may be stabilized in the conventional manner with suitable stabilizers, for example, ascorbic acid, gentisic acid or salts of these acids, or it may comprise other auxiliary agents, for example, fillers, such as glucose, lactose, mannitol, and the like.

The invention will now be described in greater detail with reference to the following specific Examples.

Example I

Synthesis of 6-(N-(Biotinylsulfoxide)-p-aminobenzyl)-1,4,8,11-tetraazaundecane(6)(Fig. 1)

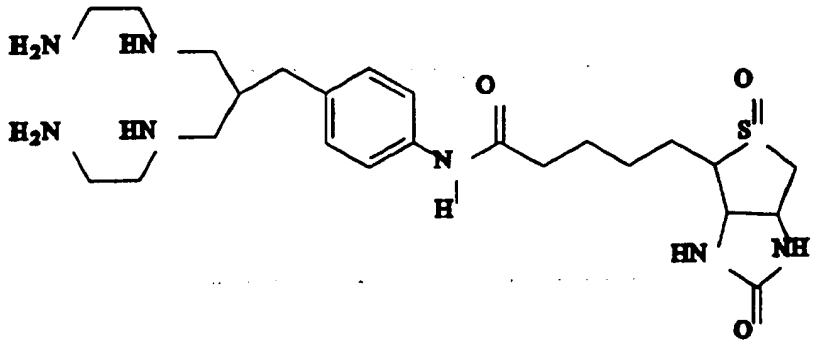


Fig. 1. Biotinsulfoxide-N_4

Biotinsulfoxide is synthesized according to Melville (D.B. Melville, J. Biol. Chem., 1954, 208, 495). The sulfoxide exists in two isomeric forms:α-(+)-biotinsulfoxide and β-(-)-biotinsulfoxide (Fig. 2)

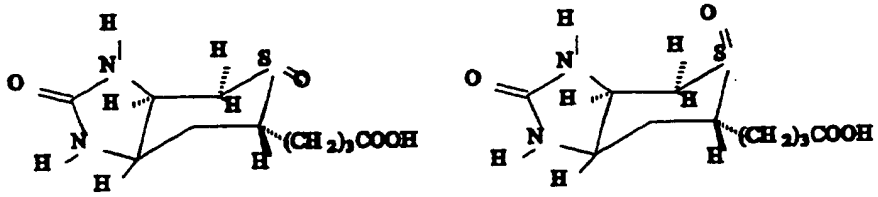


Fig. 2. Two isomeric forms of biotinsulfoxide

They can be separated by fractional crystallization and/or silica gel chromatography.

6-(p-Nitro-benzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane (1)

Four mMol (1.07 g) **p-nitrobenzylmalonic acid diethylester** (G. Ruser et al., Bioconj. Chem. 1990, 2, 345) is suspended in 40 ml methanol; 5.3 ml (80 mmol) ethylenediamine are added and the mixture is heated under reflux for 12 h. Solvent and excess ethylenediamine are evaporated and the crude product purified by silica gel chromatography ($CHCl_3$/MeOH/NH_3(25%) = 5:5:1, R_f= 0.3). Yield 1.4 g.

MS (FAB) m/z (relative intensity): 324 (MH^+, 100); ^{1}H-NMR (300 MHz; d_6-DMSO). 8.14 (d, 2H,

o-Ar-H-), 8.04 (t, 2H, CH_2-NH-CO), 7.46 (d, 2H, m-Ar-H), 3.45 (t, 1H, CH-CH_2-Ar), 3.15 (d, 2H, CH_2-Ar), 3.05 (m, 4H, CH_2-NH-), 2.50 (m, 4H, CH-NH_2); ^{13}C-NMR (90 MHz, d_6-DMSO): 168.25 (2C, C=O), 147.71 (1C, C(Ar)-NO_2), 145.86 (1C, CH_2-C(Ar)), 129.95 (2C, Ar), 123.06 (2C, Ar), 54.00 (1C, CH-CH_2-Ar), 41.93 (2C, CH_2-NH(CO)), 40.86 (2C, CH_2-NH_2), 34.48 (1C, CH_2-Ar).

Elemental analysis corresponds to $C_{14}H_{21}N_5O_4$

6-(p-Nitrobenzyl)-1,4,8,11-tetraazaundecane (2)

The crude (**1**) (480 mg, 1,48 mmol) is reacted at RT with 1M BH_3/THF solution (30 ml, 20 eq) and the reaction mixture is refluxed for 60 h. For decomposing the excess BH_3, MeOH is added dropwise with caution to the reaction mixture, which is precooled at 0° C. The MeOH is evaporated in vacuum and the residue is suspended in EtOH and sonicated for several minutes. The undissolved part is filtered off and HCl gas is introduced for 20 min to the filtrate. The formed precipitate is collected and further purified by column chromatography (SiO_2;$CHCl_3$/MeOH/25% NH_3 5/5/2). The resulting purified amine is dissolved in EtOH and the corresponding hydrochloric salt is obtained as a white solid by passing HCl gas to this solution (460 mg). Alternatively, the precipitate is directly recrystallized from HCl gas saturated MeOH, without previous purification by chromatography.

Yield = 70%; Rf: 0.36 (SiO_2; $CHCl_3$/MeOH/25% NH_3 5/5/2); MS (FAB) m/z (relative intensity): 296 (MH^+, 100); ^{1}H-NMR (360 MHz, d_6-DMSO) (hydrochloride): 8.21 (d, 2H;0-Ar-NO_2), 7.68 (d, 2H, m-Ar-NO_2), 3.50-264 (m, 15H); ^{13}C-NMR(90 MHz, d_6-DMSO): 146.29 (2C, C(Ar)-NO_2, CH_2-Ar), 130.46 (2C, Ar), 123.36 (2C, Ar), 47.56, 44.68, 35.04, 34.97.

The elemental analysis is consistent with $C_{14}Cl_4H_{20}N_5O_2$.

N,N,N',N'''-Tetrabutyloxycarbonyl-6-(p-Nitrobenzyl)-1,4,8,11-tetraazaundecane (3)

1.5 g (3.5 mMol) **(2)** is suspended in 5 ml dioxane and 1N NaOH is added until pH12. At ice temperature 4.5 ml (21 mMol/6 eq) di-tert-butyl-dicarbonate in 30 ml dioxane and 40 ml 1N NaOH are added. The mixture is stirred at RT over night. To this mixture 100 ml diethylether and 50 ml H_2O are added. The ether layer is separated and extracted four times with 30 ml water, dried with Na_2SO_4 and evaporated. The resulting oil cannot be crystallized but shows a structure confirming FAB-MS (m/z: 696) and is pure on TLC (Silica gel, RF = 0.5, acetic acid ethyl ester: hexane = 9:1). Yield: 2.2 g, 90%. FAB-MS: m/z: 696. ^{1}H NMR and IR are consistent with the structure.

N,N',N",N'"-Tetrabutyloxycarbonyl-6-(p-aminobenzyl)-1,4,8,11-tetraazaundecane (4)

1.3 g (1.9 mmol) **(3)** is dissolved in 40 ml 80% MeOH; 150 mg Pd/C (10%) are added and hydrogenation is performed for 3 h at room temperature. The catalyst is removed by filtration, the mixture evaporated and pure product obtained by silica gel chromatography (Silica gel 60, 32 cm x 2 cm, eluent: hexane: acetic acid ethylester = 2:3). Yield 0,53 g (43%). The compound is pure on TLC and HPLC and is characterized by FAB-MS (m/z = 666) and ^{1}H-NMR.

6-(N-(biotinylsulfoxide)-p-amino-benzyl)-N,N",N'"-tetrabutyloxycarbonyl-1,4,8,11-tetraazaundecane (5)

34.4 mg α-(+)-biotinsulfoxide are dissolved in 700 µl DMF; 42.5 mg HATU (O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorphosphate) and 23 µl diisopropylethylamine (Hünigbase) are added and the mixture left at room temperature for 10 min. Afterwards 88 mg of **(4)** and 23 µl Hünigbase in 1 ml DMF are added. Stirring is continued for 20 min. The reaction mixture is added to 2 ml ethylacetate and 2 ml 5% $NaHCO_3$ solution. The organic layer is washed twice with sodium bicarbonate and saturated sodium chloride solution, dried with sodium sulfate, evaporated and crystallized from diethylether/isopropylether/hexane. Yield: 96 mg (80%). The product is chromatographically pure and characterized by FAB-MS (m/z = 908).

6-(N-(biotinylsulfoxide)-p-aminobenzyl)-1,4,8,11-tetraazaundecane (6) (Fig. 1)

96 mg **(5)** is dissolved in 2 ml trifluoracetic acid: thioanisole: water = 96:2:2 and left for 2 h at room temperature. Thereafter the product is precipitated by the addition of 2 ml diethylether and dried under high vacuum. Yield: 96 mg. To remove small impurities and remaining thioanisole the compound is purified by C18-HPLC. FAB-MS: m/z = 508.

Example II

^{99m}Tc-Labelling of (6)

Fifty µg of **(6)** obtained as described in Example I, are dissolved in 300 µl of an aqueous

solution of sodium citrate dihydrate (5 mg). To this solution 200-400 µl (1000-2000 MBq) $^{99m}TcO_4^-$ generator eluate and 20 µl (20 µg) of a freshly prepared N_2-purged $SnCl_2$. 2 H_2O solution (10mg/10 ml 0.1 M HCl) are added. The pH is adjusted to 10 with naOH. Incubation is done at room temperature for 30 min. Complexing yield is better than 97%. On a Hamilton PRP-1 (10 µm, 4.1 x 150 mm) column and isocratic elution with 100% 10 mM phosphate buffer (pH 7, 0-5 min), followed by a linear gradient (5-10 min; 20% MeCN/80% phosphate buffer) and isocratic elution up to 15 min the ^{99m}Tc complex elutes at 10'04" (flow: 1.5 ml min). Under the same conditions (^{99m}Tc)6-(p-aminobenzyl)-1,4,8,11-tetraazaundecane elutes at 8'45". The complex is stable in citrate and phosphate buffer for at least 10 h.

Example III

Binding of (^{99m}Tc)-(6) to avidin

(^{99m}Tc)-(6), obtained as described in Example II, is loaded onto a column of avidin coupled to beaded agarose which was preconditioned with 3 times 1.5 ml phosphate buffer (pH 8.9). The column is washed with 5 times 1.5 ml phosphate buffer. Activity retained on the column is 97±3% (n = 3). If the column is presaturated with cold biotin less than 2% of the activity is retained. So the conclusion is that the binding to avidin is specific.

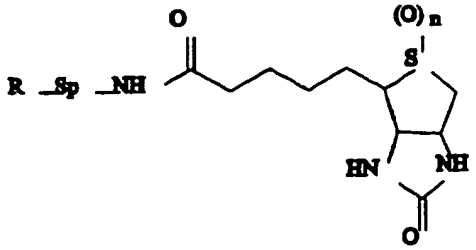
Example IV

Stability of (^{99m}Tc)-(6) in human serum

Biotinidase is an enzyme found in different organs and in human serum which functions as a biotin-amide amidohydrolase; e.g. it hydrolyzes (N-(d-biotinyl)-p-aminobenzoic acid and biocytin. The stability of the amide bond in fresh human serum is studied. Within 4 h of incubation the amount of intact **(^{99m}Tc)-(6)**, obtained according to Example II, decreases by about 15±2% which is by far superior to the corresponding conjugate with biotin. Considering in fact that the optimal time of scintigraphy with a ^{99m}Tc-labelled biotin conjugate is within 2 h post injection, the stability of **^{99m}Tc(6)** in human serum is adequate for human studies.

Claims

1. A labelled biotin compound, wherein said biotin compound is represented by the general formula



(I)

wherein:

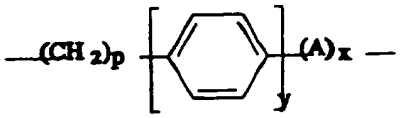
n is 1 or 2,

R is a chelating group for chelating a metal atom, and

Sp is a spacing group having at least 4 atoms spacing NH from R; and

wherein said biotin compound is labelled with a metal atom selected from (a) the group consisting of the radioactive isotopes ^{99m}Tc, ^{203}Pb, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{72}As, ^{111}In, ^{113m}In, ^{114m}In, ^{97}Ru, ^{62}Cu, ^{64}Cu, ^{52}Fe, ^{52m}Mn, ^{51}Cr, ^{186}Re, ^{188}Re, ^{77}As, ^{90}Y, ^{67}Cu, ^{169}Er, ^{117m}Sn, ^{121}Sn, ^{127}Te, ^{142}Pr, ^{143}Pr, ^{198}Au, ^{199}Au, ^{149}Tb, ^{161}Tb, ^{109}Pd, ^{165}Dy, ^{149}Pm, ^{151}Pm, ^{153}Sm, ^{157}Gd, ^{166}Ho, ^{172}Tm, ^{169}Yb, ^{175}Yb, ^{177}Lu, ^{105}Rh and ^{111}Ag or (b) the group consisting of the paramagnetic metal atoms Cr, Mn, Fe, Co, Ni, Cu, Pr, Nd, Sm, Yb, Gd, Tb, Dy, Ho and Er; said metal atom being attached to the biotin compound by means of said chelating group.

2. A labelled biotin compound as claimed in claim 1, wherein said spacing group Sp is a group of the general formula



(II)

wherein A is a biradical of the formula

-NH-$(CH_2)_m$- or -NH-C(=X)-$(CH_2)_x$-Y-$(CH_2)_m$-C(CO_2H)H-

wherein x and y are each independently 0 or 1;

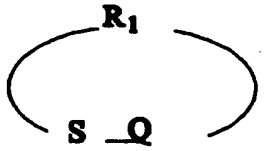
m is an integer from 1 to 4;

p is an integer from 0 to 4;

X is O or S; and

Y is NH, CO or S.

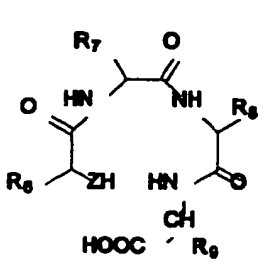
3. A labelled biotin compound as claimed in claim 1 or 2, wherein said chelating group R is selected from the group consisting of $N_pP_qS_{(4+q)}$ tetradentate chelating agents, wherein t + q = 2-4, or groups derived from ethylene diamine tetra-acetic acid (EDTA), diethylene triamine penta-acetic acid (DTPA), cyclohexyl 1,2-diamine tetra-acetic acid (CDTA), ethyleneglycol-0,0'-bis(2-aminoethyl)-N,N,N',N'-tetra-acetic acid (EGTA), N,N-bis(hydroxybenzyl)-ethylenediamine-N,N'-diacetic acid (HBED), triethylene tetramine hexa-acetic acid (TTHA), 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tri and tetra-acetic acid (DO3A and DOTA), 1,4,7,10-tetraazacyclotridecane-N,N',N'',N'''-tri and tetra-acetic acid (TRI3A and TRITA), hydroxyethyldiamine triacetic acid (HEDTA), 1,4,8,11-tetra-azacyclotetradecane-N,N',N'',N'''-tetra-acetic acid (TETA), substituted DTPA, substituted EDTA, or from deferoxamine, or from a compound of the general formula



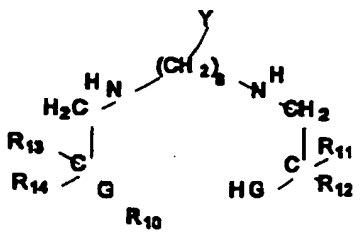
(III)

wherein R_1 is a branched or non-branched, optionally substituted hydrocarbyl radical, which may be interrupted by one or more hetero-atoms selected from N, O and S and/or by one or more NH groups, and
Q is a group which is capable of reacting with an amino group of the peptide and which is preferably selected from the group consisting of carbonyl, carbimidoyl, N-(C_1-C_5)alkylcarbimidoyl, N-hydroxycarbimidoyl and N-(C_1-C_5)alkoxycarbimidoyl.

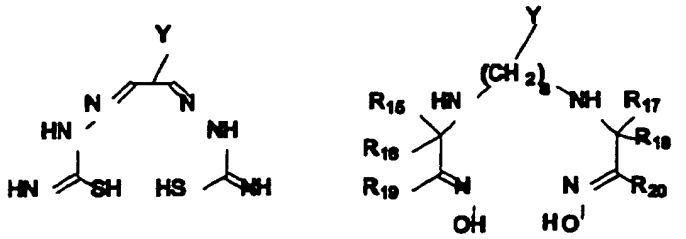
4. A labelled biotin compound as claimed in claim 3, wherein said chelating group R is a group is selected from



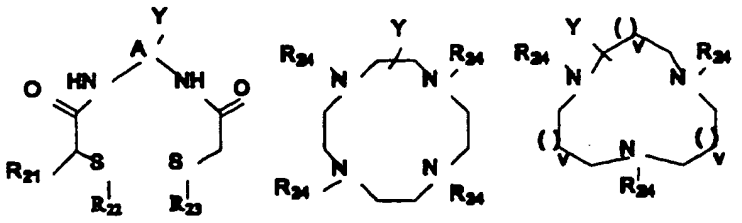
(IV)



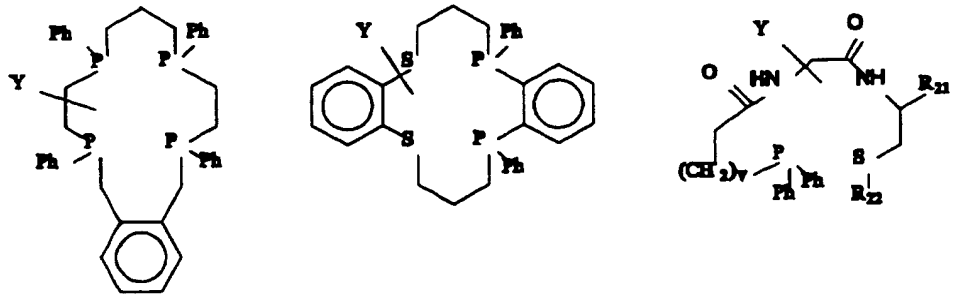
(V)



(VI) (VII)



(VIII) (IX) (X)



(XI) (XII) (XIII)

wherein:

R_6-R_{20} are each individually hydrogen atoms or (C_1-C_4)alkyl groups, with the proviso that at least one of C_6 to C_9 is the symbol Y;

R_{21} is a hydrogen atom or a CO_2(C_1-C_4)alkyl group;

R_{22} and R_{23} are each individually (C_1-C_4)alkylcarbonyl, benzoyl or benzyl groups;

v is 0 or 1;

s is 2 or 3;

R_{24} is CH_2COOH or a functional derivative thereof;

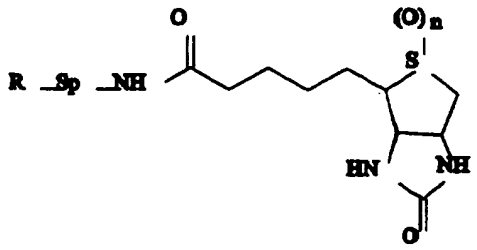
A is (C_1-C_4)alkylene, if desired substituted with CO_2alkyl, $CH_2COalkyl$, $CONH_2$, $CONHCH_2CO_2alkyl$; phenylene, phenylene substituted by CO_2alkyl, wherein the alkyl groups have 1 to 4 carbon atoms;

G is NH or S;

Y is a valence bond or a functional group capable of binding with the spacing group; and
Z is S or O.

5. A method of preparing a labelled biotin compound as claimed in claim 1, characterized in that a biotin compound of the general formula I, as shown in claim 1, wherein the symbols have the meanings given in claim 1, is reacted with a metal atom as defined in claim 1 in the form of a salt or of a chelate bound to a comparatively weak chelator, in order to form a complex.

6. A biotin compound to be used for the method according to claim 5, having the general formula



(I)

wherein:

n is 1 or 2,
R is a chelating group for chelating a metal atom, and
Sp is a spacing group having at least 4 atoms spacing NH from R.

7. A pharmaceutical composition, comprising in addition to a pharmaceutically acceptable carrier material and, if desired, at least one pharmaceutically acceptable adjuvant, as the active substance a labelled biotin compound as claimed in claim 1, 2, 3 or 4.

8. A method for detecting and localizing tumours in the body of a warm-blooded living being, which comprises (i) administering to said being a composition comprising (strept)avidin conjugated with a polypeptide having a selective affinity to said tumour, (ii) thereupon, after avidination of said tumour, administering to said being a composition comprising, in a quantity sufficient for external imaging, a labelled biotin compound as claimed in claim 1, 2, 3 or 4, said biotin compound being labelled with (a) a radioactive metal isotope selected from the group consisting of ^{99m}Tc, ^{203}Pb, ^{67}Ga, ^{68}Ga, ^{72}As, ^{111}In, ^{113m}In, ^{97}Ru, ^{62}Cu, ^{64}Cu, ^{52}Fe, ^{52m}Mn and ^{51}Cr, or (b) with a paramagnetic metal atom selected from the group consisting of Cr, Mn, Fe, Co, Ni, Cu, Pr, Nd, Sm, Yb, Gd, Tb, Dy, Ho and Er, and

(iii) finally subjecting said being to external imaging to determine the targeted sites in the body of said being in relation to the background activity.

9. A method for detecting and localizing tumours in the body of a warm-blooded living being, which comprises (i) administering to said being a composition comprising a biotinylated polypeptide having a selective affinity to said tumour, (ii) then administering a composition comprising (strept)avidin, (iii) thereupon, after avidination of said tumour, administering to said being a composition comprising, in a quantity sufficient for external imaging, a labelled biotin compound as claimed in claim 1, 2, 3 or 4, said biotin compound being labelled with (a) a radioactive metal isotope selected from the group consisting of ^{99m}Tc, ^{203}Pb, ^{67}Ga, ^{68}Ga, ^{72}As, ^{111}In, ^{113m}In, ^{97}Ru, ^{62}Cu, ^{64}Cu, ^{52}Fe, ^{52m}Mn and ^{51}Cr, or (b) with a paramagnetic metal atom selected from the group consisting of Cr, Mn, Fe, Co, Ni, Cu, Pr, Nd, Sm, Yb, Gd, Tb, Dy, Ho and Er, and (iv) finally subjecting said being to external imaging to determine the targeted sites in the body of said being in relation to the background activity.

10. A method of intraoperatively detecting and localizing tumours in the body of a warm-blooded living being, which comprises (i) administering to said being a composition comprising (strept)avidin conjugated with a polypeptide having a selective affinity to said tumour, (ii) thereupon, after avidination of said tumour, administering to said being a composition comprising, in a quantity sufficient for detection by a gamma detecting probe, a labelled biotin compound as claimed in claim 1, 2, 3 or 4, said biotin compound being labelled with ^{161}Tb, and (iii) finally, after allowing the active substance to be bound by and taken up in said tumour and after blood clearance of radioactivity, subjecting said being to a radioimmunodetection technique in the relevant area of the body of said being, by using a gamma detecting probe.

11. A method of intraoperatively detecting and localizing tumours in the body of a warm-blooded living being, which comprises (i) administering to said being a composition comprising a biotinylated polypeptide having a selective affinity to said tumour, (ii) then administering a composition comprising (strept)avidin, (iii) thereupon, after avidination of said tumour, administering to said being a composition comprising, in a quantity sufficient for detection by a gamma detecting probe, a labelled biotin compound as claimed in claim 1, 2, 3 or 4, said biotin compound being labelled with ^{161}Tb, and (iv) finally, after allowing the active substance to be bound and taken up in said tumour and after blood clearance of radioactivity, subjecting said being to a radioimmunodetection technique in the relevant area of the body of said being, by using a gamma detecting probe.

12. A method for the therapeutic treatment of tumours in the body of a warm-blooded living being, which comprises (i) administering to said being a composition comprising (strept)avidin conjugated with a polypeptide having a selective affinity to said tumour, (ii) thereupon, after avidination of said tumour, administering to said being a composition comprising,, in a quantity effective for combating or controling tumours, a labelled biotin compound as claimed in claim 1, 2, 3 or 4, said biotin compound being labelled with a metal isotope selected from the group consisting of ^{114m}In, ^{186}Re, ^{188}Re, ^{77}As, ^{90}Y, ^{66}Ga, ^{67}Cu, ^{169}Er, ^{117m}Sn, ^{121}Sn, ^{127}Te, ^{142}Pr, ^{143}Pr, ^{198}Au, ^{199}Au, ^{149}Tb, ^{161}Tb, ^{109}Pd, ^{165}Dy, ^{149}Pm, ^{151}Pm, ^{153}Sm, ^{157}Gd, ^{159}Gd, ^{166}Ho, ^{172}Tm, ^{169}Yb, ^{175}Yb, ^{177}Lu, ^{105}Rh and ^{111}Ag.

13. A method for the therapeutic treatment of tumours in the body of a warm-blooded living being, which comprises (i) administering to said being a composition comprising a biotinylated polypeptide having a selective affinity to said tumour, (ii) then administering a composition comprising (strept)avidin, (iii) thereupon, after avidination of said tumour, administering to said being a composition comprising, in a quantity effective for combating or controling tumours, a labelled biotin compound as claimed in claim 1, 2, 3 or 4, said biotin compound being labelled with a metal isotope selected from the group consisting of ^{114m}In, ^{186}Re, ^{188}Re, ^{77}As, ^{90}Y, ^{66}Ga, ^{67}Cu, ^{169}Er, ^{117m}Sn, ^{121}Sn, ^{127}Te, ^{142}Pr, ^{143}Pr, ^{198}Au, ^{199}Au, ^{149}Tb, ^{161}Tb, ^{109}Pd, ^{165}Dy, ^{149}Pm, ^{151}Pm, ^{153}Sm, ^{157}Gd, ^{159}Gd, ^{166}Ho, ^{172}Tm, ^{169}Yb, ^{175}Yb, ^{177}Lu, ^{105}Rh and ^{111}Ag.

14. A kit for preparing a radiopharmaceutical composition, comprising (a) a composition comprising (strept)avidin conjugated with a polypeptide having a selective affinity to tumours, (b) a biotine compound of the general formula I, shown in claim 1, wherein the symbols have the meanings given in claim 1, to which compound, if desired, an inert pharmaceutically acceptable carrier and/or formulating agents and/or adjuvants is/are added, (c) a solution of a salt or chelate of a metal isotope selected from the group consisting of ^{203}Pb, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{72}As, ^{111}In, ^{113m}In, ^{114m}In, ^{97}Ru, ^{62}Cu, ^{99m}Tc, ^{186}Re, ^{188}Re, ^{64}Cu, ^{52}Fe, ^{52m}Mn, ^{51}Cr, ^{77}As, ^{90}Y, ^{67}Cu, ^{169}Er, ^{117m}Sn, ^{121}Sn, ^{127}Te, ^{142}Pr, ^{143}Pr, ^{198}Au, ^{199}Au, ^{149}Tb, ^{161}Tb, ^{109}Pd, ^{165}Dy, ^{149}Pm, ^{151}Pm, ^{153}Sm, ^{157}Gd, ^{166}Ho, ^{172}Tm, ^{169}Yb, ^{175}Yb, ^{177}Lu, ^{105}Rh and ^{111}Ag, and (d) instructions for use with a prescription for reacting the ingredients present in the kit.

15. A kit for preparing a radiopharmaceutical composition, comprising (a) a composition comprising a biotinylated polypeptide having a selective affinity to tumours, (b) a composition comprising (strept)avidine, (c) a biotine compound of the general formula I, shown in claim 1, wherein the symbols have the meanings given in claim 1, to which

compound, if desired, an inert pharmaceutically acceptable carrier and/or formulating agents and/or adjuvants is/are added, (d) a solution of a salt or chelate of a metal isotope selected from the group con-sisting of ^{203}Pb, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{72}As, ^{111}In, ^{113m}In, ^{114m}In, ^{97}Ru, ^{62}Cu, ^{99m}Tc, ^{186}Re, ^{188}Re, ^{64}Cu, ^{52}Fe, ^{52m}Mn, ^{51}Cr, ^{77}As, ^{90}Y, ^{67}Cu, ^{169}Er, ^{117m}Sn, ^{121}Sn, ^{127}Te, ^{142}Pr, ^{143}Pr, ^{198}Au, ^{199}Au, ^{149}Tb, ^{161}Tb, ^{109}Pd, ^{165}Dy, ^{149}Pm, ^{151}Pm, ^{153}Sm, ^{157}Gd, ^{166}Ho, ^{172}Tm, ^{169}Yb, ^{175}Yb, ^{177}Lu, ^{105}Rh and ^{111}Ag, and (e) instructions for use with a prescription for reacting the ingredients present in the kit.

16. A kit for preparing a radiopharmaceutical composition, comprising (a) a composition comprising (strept)avidin conjugated with a polypeptide having a selective affinity to tumours, (b) a biotine compound of the general formula I, shown in claim 1, wherein the symbols have the meanings given in claim 1, to which compound, if desired, an inert pharmaceutically acceptable carrier and/or formulating agents and/or adjuvants is/are added, (c) a reducing agent, and, if desired, a chelator, and (d) instructions for use with a prescription for reacting the ingredients of the kit with ^{99m}Tc in the form of a pertechnetate solution or with ^{186}Re or ^{188}Re in the form of a perrhenate solution.

17. A kit for preparing a radiopharmaceutical composition, comprising (a) a composition comprising a biotinylated polypeptide having a selective affinity to tumours, (b) a composition comprising (strept)avidine, (c) a biotine compound of the general formula I, shown in claim 1, wherein the symbols have the meanings given in claim 1, to which compound, if desired, an inert pharmaceutically acceptable carrier and/or formulating agents and/or adjuvants is/are added, (c) a reducing agent, and, if desired, a chelator, and (d) instructions for use with a prescription for reacting the ingredients of the kit with ^{99m}Tc in the form of a pertechnetate solution or with ^{186}Re or ^{188}Re in the form of a perrhenate solution.

INTERNATIONAL SEARCH REPORT

Inv ional Application No
PCT/US 96/14883

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K51/04 A61K49/00 //A61K103:10,A61K121:00,A61K123:00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BIOCHEM. J., 1990, VOL. 268, NO. 3, PAGE(S) 611-13, XP000564516 GARLICK R K ET AL: "Dissociative binding of.alpha.- and.beta.-sulfoxides of biotinylamidoethyl-3-(4-hydroxy-3-[125I]iodophenyl)propionamide to avidin" see abstract * see paragraph introduction * * see paragraph Results and discussion * see figure 1 --- -/--	1-17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance

'E' earlier document but published on or after the international filing date

'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 January 1997	11. 02. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Dullaart, A

Form PCT ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 96/14883

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 8-13
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1-17
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

In view of the large number of compounds which are defined by the wording of the claims, the search has been performed on the general idea and compounds mentioned in the examples of the description.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NUCL. MED. BIOL., 1994, VOL. 21, NO. 7, PAGE(S) 935-940, XP002024150 JEONG J.M. ET AL: "Application of high affinity binding concept to radiolabel avidin with Tc-99m labeled biotin and the effect of pI on biodistribution" see abstract * see paragraph Introduction * * Scheme 1 * * see paragraph Materials and methods *	1,3,5-17
Y	INT J RAD APPL INSTRUM [B], 1991, VOL. 18, NO. 7, PAGE(S) 719-26, XP000235562 VIRZI F ET AL: "NEW INDIUM-111 LABELED BIOTIN DERIVATIVES FOR IMPROVED IMMUNOTARGETING" see abstract * see paragraph Experimental section * see figures 1-3,6 see tables 1-3	1-17
Y	ANGEW. CHEM. INT. ED. ENGL., 1992, VOL. 31, NO. 11, PAGE(S) 1507-1509, XP000325384 KOCH P. ET AL: "99mTc labeled biotin conjugate in a tumor 'pretargeting' approach with monoclonal antibodies" see figure 2 see table 1 see page 1508	1,2,4-17
Y	BIOCHEMISTRY, 1970, VOL. 9, NO. 17, PAGE(S) 3285-93, XP002024151 GRIESSER R ET AL: "Stability and structure of binary and ternary metal ion complexes with biocytin, the sulfoxide and sulfone, N-acetyl-L-lysine, and L-alanine" see abstract see tables 1,2 * see paragraph Conclusions *	1-17
P,X	US 5 541 287 A (E.K. YAU ET AL.) 30 July 1996 see example 18C	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 96/14883

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-5541287	30-07-96	US-A-	5283342	01-02-94
		CA-A-	2177136	08-06-95
		EP-A-	0736035	09-10-96
		WO-A-	9515335	08-06-95
		CA-A-	2134239	23-12-93
		EP-A-	0646019	05-04-95
		JP-T-	7507804	31-08-95
		WO-A-	9325240	23-12-93
		US-A-	5578287	26-11-96